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14. ABSTRACT

This application focuses on enhancing cancer care by developing non-invasive techniques to determine better biomarkers to improve diagnostic specificity and decrease the number of negative biopsies, and also as markers of response with novel targeted agents such as Trastuzumab and Bevacizumab. Last year, we optimized lactate sequence (SS1-SelMQC) using higher order binomial pulses with better lipid suppression compared to original SS-SelMQC and started working on breast tumors. In this year, we completed data collection of lactate MR spectroscopy data of in vivo breast tumors with different expression levels of ER, PR, HER-2 as a function of Herceptin and Avastin. We completed quantification of 1D spectra and made significant progress in 2D quantification to explain tumor heterogeneity, an important step towards understanding treatment response. Presently, we are working on completing the data analysis of all 2D data analysis simultaneously along with the histological correlation.

15. SUBJECT TERMS
None provided.

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Introduction

The main objective of the proposal is developing and evaluating a more effective method of detecting lactate (Lac) non-invasively by magnetic resonance spectroscopy (MRS) techniques. Lac is present in very small quantities (milli moles) compared to lipid (Lip) (moles) and water and the signal of Lac occurs in the same position as Lip, thereby making it very difficult to see. Treatment of breast cancers with novel targeted agents such as Trastuzumab and Bevacizumab have led to significant gains, although the drugs can be toxic. Breast tumors are usually sensitive to many drugs but subsequently develop resistance. There is strong interest in applying drugs that interfere with angiogenesis and signaling pathways related to breast cancer growth and metastasis. Low extracellular pH and high Lac levels were shown to be indicators of metastatic risk in breast cancer xenografts. Elevated Lac in biopsy samples was shown to correlate with increased risk of metastasis and poor patient survival in different aggressive cancers, while a decrease Lac levels observed in tumor response to radiation and chemotherapy. Therefore, non-invasive measurement of Lac may be an additional characteristic marker for breast cancer; it may improve diagnostic specificity, serve as an early marker of tumor response, and provide functional information about prognosis.

Quantification of Lac using MRS is a non-invasive powerful tool for early cancer diagnosis and treatment monitoring. Last year, we published a manuscript showing lactate as a biomarker for metastasis in animal tumors (1). Recently we have submitted a study demonstrating relationships between LDH-A, Lac and metastases in 4T1 breast tumors to clinical cancer research (Manuscript is under revision). During this project period, we have calculated the lactate concentration [Lac] in breast mammary tumors with different ER/PR, and HER2 status. We also compared [Lac] between slow growing tumors with fast growing tumors. We completed the collection of MR spectroscopy scans to study breast tumors treated with Avastin and Herceptin. We are in the process of completing all unfinished analysis of treatment data along with the histologic image correlations to finalize the manuscripts for submission.

Body

We have made progress in completing 2D data collection of lactate for treatment studies of breast tumors as a function of targeted drugs. We have also completed 1D whole and slice lactate MR data analysis and currently working on 2D data analysis and histology correlations. As mentioned in our last report, we also finished uncompleted 2D lactate data analysis from aggressive tumors such as MDA231 and MDA435 as a function of increasing tumor volumes.

As part of our research design, we will determine if [Lac] is a marker of sensitivity to novel targeted drugs used in MDA435 and MDA231 models and to test if lactate concentrations may predict tumor aggressiveness using various metastatic and non-metastatic breast cancers. We used MCF-7, BT-474, MDA-MB-231 and MDA-MB-435 tumors. We have calculated the T1 and T2 relaxation times for lactate quantification purpose and further investigated to study any significant differences between these tumors with respect to their different growth rates and aggressiveness. For tumor treatment, we used tumors at volumes between 150-200mm3.

Methods and Materials

Data is collected in compliance with the Institutional Animal Care and Use Committee approved protocol. Cells (5×10^6) were implanted into the mammary fat pad (MFP) of 4-6-week-old female athymic nu/nu mice. We used MCF7, BT474, MDA-MB-231, and MDA-MB-435 cell lines. Two days prior to implantation, estrogen pellets inserted in MCF-7 and BT-474 group mice. Tumor growth became evident on visual inspection about 10 days after implantation into the MFP. For MR studies, tumor bearing mice were anesthetized with isoflurane (1.0-2.5%) and compressed air. MRS treatment studies were conducted for tumors in the range of 100-200 mm^3. Tumor bearing mice were typically studied 3-4 times.

Experiments were done on 4.7 T Bruker horizontal bore system. Mice were anesthetized with a mixture of isoflurane (1.5-2.5%) in air and placed inside a home-built MR animal holder. The respiration and temperature were monitored using an MR compatible animal monitoring and gating system. The tumor was placed inside a 2 turn home built 10-11 mm (upto 400 mm^3) diameter tuned coil. Temperature was maintained at 37°C by blowing warm air through the bore of the magnet. The magnet was shimmed to a FWHM line width of less than 50 Hz for the proton signal. SS1-SelMQC parameters follows the published report (2,3) with the spectral-selective RF pulses replaced with higher order binomial bp1, bp2 and bp3 RF pulses blocks $[(\pi/16)_{-x} - \Delta_1 - (3\pi/16)_x - \Delta_1 - (3\pi/16)_{-x}) - \Delta_1 - (\pi/16)_x]$, $[(\pi/16)_x - \Delta_1 - (3\pi/16)_x - \Delta_1 - (3\pi/16)_x) - \Delta_1 - (\pi/16)_x]$, and $[(\pi/8)_{-x} - \Delta_2 - (3\pi/8)_x - \Delta_2 - (3\pi/8)_{-x} - \Delta_2 - (\pi/8)_x]$. For slice localization, we used 1ms sinc3 pulse.

MR spectroscopy acquisition parameters: 11.25°, 22.5°,45°, 33.75°, 67.5° and 90° pulse flip angle three-lobe sinc shaped pulses with 200 μs and 400 μs pulse duration, a pulse repetition of 3 s and spectral width of 12.5 ppm. Transmitter is set at CH frequency of Lac and all other experimental parameters are chosen from ref (1). The ZQ $\rightarrow$DQ coherence transfer pathway is selected with the Gsel gradients in a ratio of 0:-1:2; All 2D sets were collected using 2D SS1-SelMQC with 1^{st} pulse keeping to 90 slice selection pulse. The pulse sequence parameters for the lactate editing experiments included 512 data points, 8 averages, TR=2 s and a spectral width of 2500Hz. A matrix size of 16×16, FOV of 40 mm (1.25 x 1.25 mm in plane resolution) was used. Two-dimensional chemical shift imaging lactate maps were generated by selecting a 5 mm slice using a 1ms three-lobe Sinc pulse. The 2D CSI lactate map was visually co-registered with T_2-weighted images of 5 mm slice thickness from the center of the tumor.

T1 and T2 constants were calculated using the modified sequence presented at ISMRM (4).

Data Processing

1D data: Time domain spin echo lactate 1D data were imported the PC and were processed using matlab (Bruker). This data was fourier transformed using 2hz exponential and 1.5hz Gaussian filtering. After calculating the power spectrum, lactate peaks were fitted with a Gaussian function. The area under the lactate peak was calculated and the lactate concentration was computed using phantom substitution method using an external standard with the known concentration. The accuracy of lactate quantification of was verified using one phantom (30 mM) as the object of interest and the other phantom (15 mM) as the reference. The mean measured lactate concentration was within 4% of the nominal concentration. In vivo lactate concentration was calculated similarly.

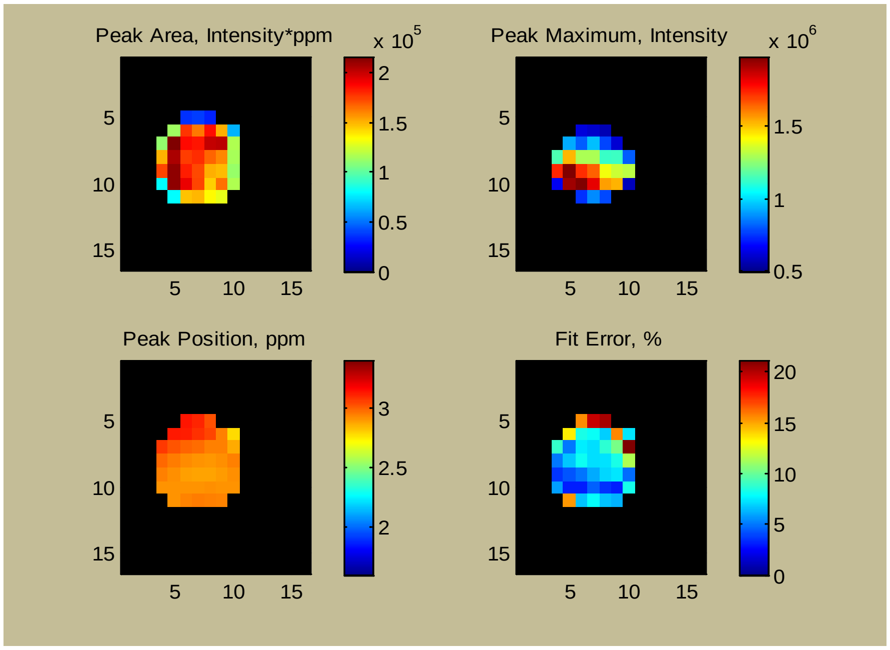

Fig. 1: Lactate peak fitting from 2D chemical shift image for quantification: peak area under peak (top left), peak maximum (top right), peak position (bottom left), and fit error (bottom right). Low fit error shows the estimate of quantification accuracy.

2D data: 2D CSI data were imported into 3DiCSI and superimposed onto T2 images. Voxel spectra were then exported in ASCII format and lactate peaks were fitted with a Gaussian function in Matlab. The area under the lactate peak was calculated and the lactate concentration was computed voxel-by-voxel using phantom substitution method.

Statistical analysis was performed using SPSS (SPSS 10.0, SPSS, Chicago, IL). The mean and standard deviations of T1 and T2 values for each tumor cell line were compared using t-test. T1 and T2 values were compared between groups of slow growing and fast growing, HER2 positive and HER2 negative, Triple negative tumors and rest of tumors.

Results:

Using SS1-SelMQC pulse sequence, T1 and T2 values were calculated for in vivo MCF, BT-474, MDA231 and MDA435 breast tumors (Table). We found no significant correlation of T1 values in tumors with different expression levels of ER, PR and HER2 levels (Fig.2). T2 values are significantly lower in triple negative tumors compared with other tumors. These factors lead to correction factors in calculating lactate concentrations. For quantification of lactate 1D and 2D data sets, external spherical phantom with 30mM lactate solution was used as a reference.

Tumor model	Mice (N)	Mean T1,s	SD T1,s	Mean T2,ms	SD T2,ms
MCF-7	4	1.88	0.33	173.75	17.25
BT-474	4	1.60	0.44	182.10	27.23
MDA-MD-231	4	1.55	0.28	124.55	41.46
MDA-MD-435	3	1.81	0.20	123.23	6.89

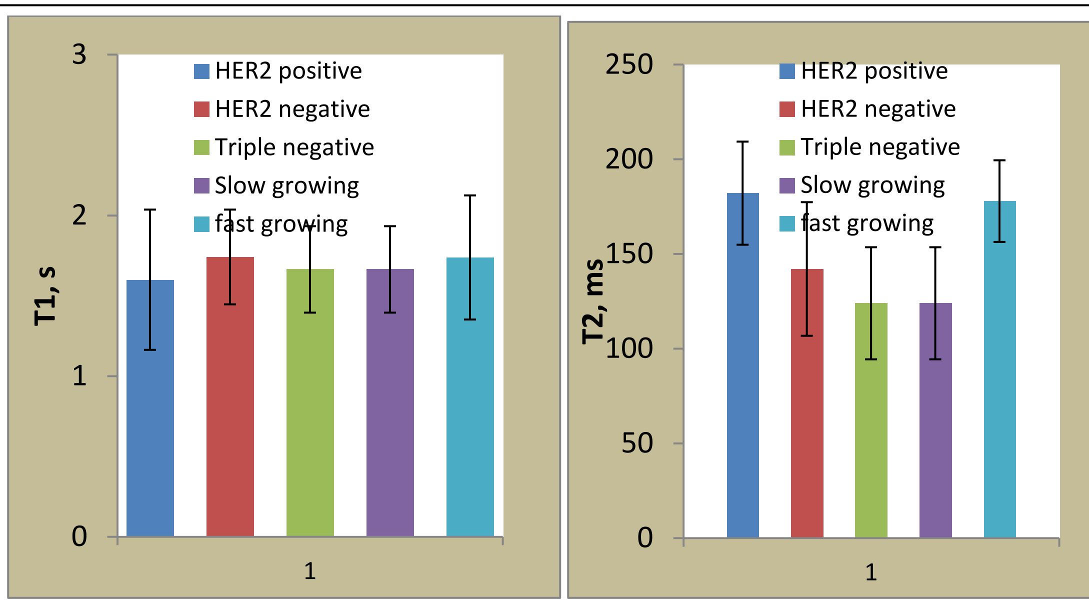

Fig. 2. T1 (left panel) and T2 (right panel) relaxation times were plotted for breast tumors with different levels of ER, PR, and HER2 tumors. There is no significant difference in T1 values. Triple negative tumors have much shorter T2 values than other tumors.

Lac spectroscopic studies of MCF-7(0-800 mm^3), MDA-MB-231(0-600 mm^3), MDA-MB-435 (0-650 mm3), BT-474 (0-900 mm^3) and analyzed the data. We collected non-localized Lac signal from 1D and slice localized 1D and 2D chemical shift imaging data.

We calculated concentrations from 1D signal using external reference phantom of 15mM lactate. For most of the 2D data sets, lactate concentrations were estimated and histology correlation needs to be completed during coming months. For all four tumor models, lactate concentrations [Lac] were calculated using external reference method. The [Lac] in MCF-7, BT-474, MDA-MB-231and MDA-MB-435 tumors were measured with respect to tumor volume (**Fig. 3**). Preliminary data was presented at a MR conference (5).

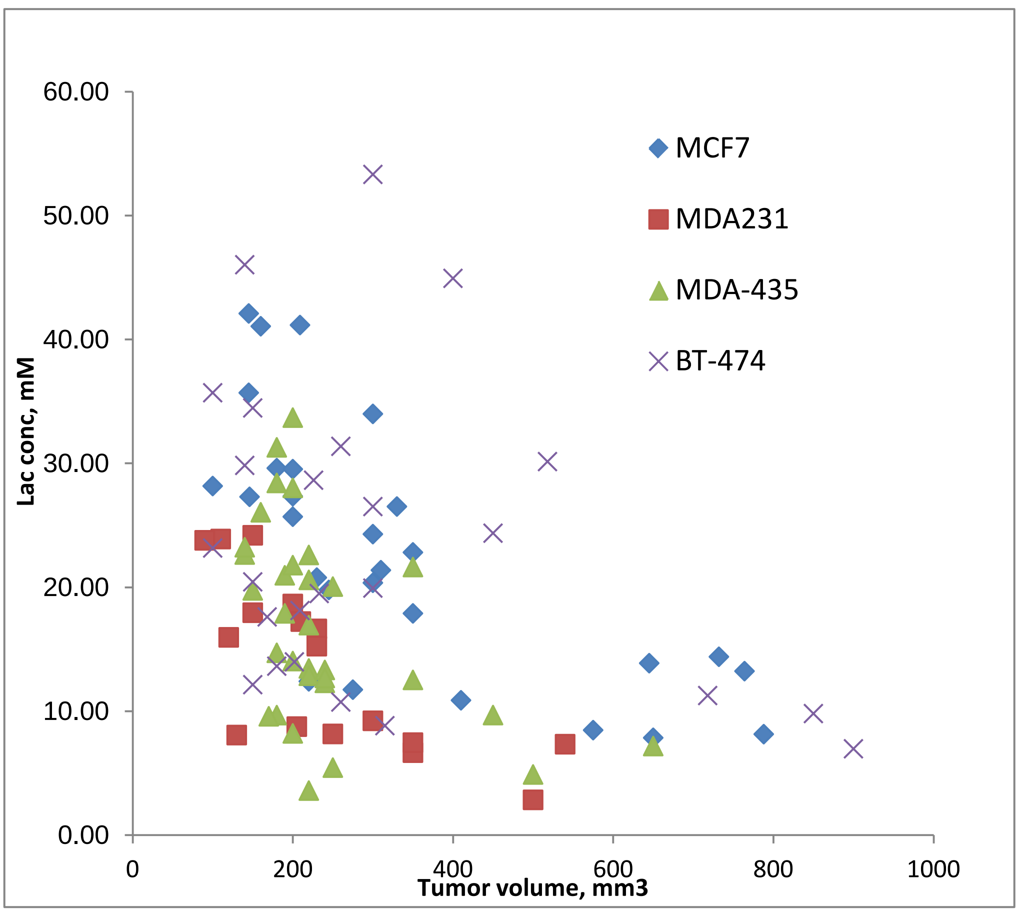

Fig. 3. Lac concentration was plotted as a function of increasing tumor volume. MCF7 and BT474 tumors have higher lactate concentrations compared with MDA231 and MDA435 tumors.

Representative 2D lactate color maps were generated for BT-474 tumor at three different tumor volumes. We are working on histology slides to correlate with these lactate maps. (<u>Manuscript is under preparation</u>).

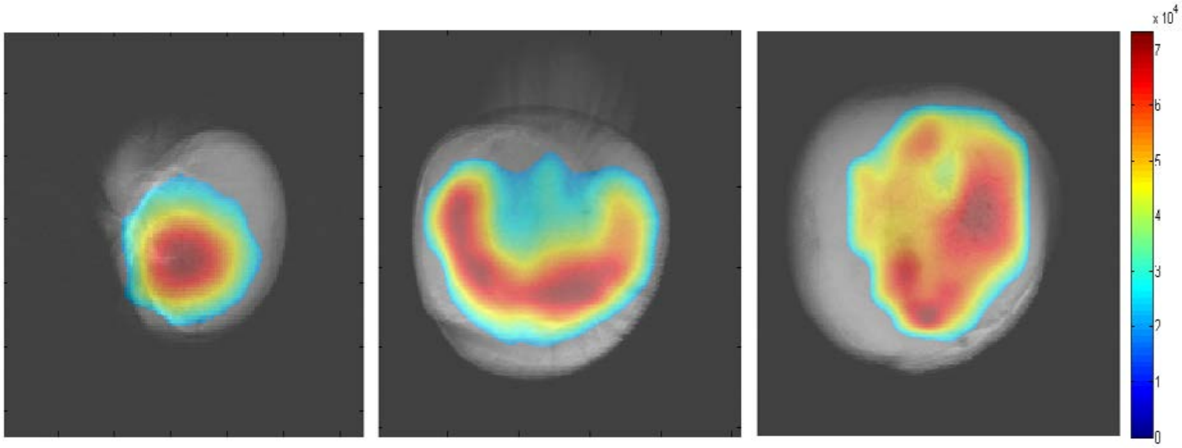

Fig. 4. Lactate area under the peak plotted as color map with increasing tumor volumes in BT-474 tumors shown by grey T2 images. Area under the peak will be converted into millimole concentrations using external reference data. Tumor volumes: (left) 200 mm3 (middle) 450 mm3 (right) 718mm3.

We have treated the breast tumors with targeted drugs. From the control data it is visible that lactate concentrations are high within tumor volumes of 150-200 mm3. So we started treating the mice at this tumor volume. Preliminary data on MDA-435 tumors treatment with Avastin demonstrated that lactate levels were decreased at the 2nd day of treatment with no visible reduction in the tumor volume (Fig. 5). Preliminary results demonstrate that lactate can be an early biomarker in treatment.

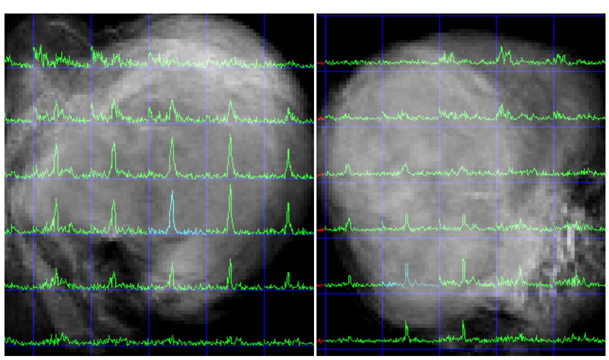

Fig. 5. 2D chemical shift imaging lactate spectra from 5mm sagittal slice within MDA435 tumor (tumor volume= 160 mm3) as a function of Avastin treatment- 0th day (left) and 2nd day (right). Reduction in the lactate levels are visible at the 2nd day of

Following the method explained in the last annual report for histology cross-sections, during this period, we will be working on rest of tumor tissue histology analysis. Finally we will be correlating the 2D lactate data using voxel-by-voxel analysis to explain tumor heterogeneity and treatment response (Manuscript is under preparation).

KEY RESEARCH ACCOMPLISHMENTS:

1. T2 relaxation constant was shorter in triple negative tumors compared with rest of tumors. Thus it may act as a marker for differentiation of these triple negative tumors.

2. Lactate concentrations were lower in MDA-231 and MDA-435 tumors compared with MCF7 and BT-474 tumors and hence [Lac] may used as a marker to differentiate fast growing tumors from slow growing tumors.

3. [Lac] seems to be an early marker for studying treatment response in MDA-435 tumors and MDA-231 tumors.

REPORTABLE OUTCOMES:

None

CONCLUSION:

Lactate concentrations [Lac] were calculated in invivo breast mammary tumors such as MCF-7, MDA-231, MDA-435, and BT-474 with increasing tumor volumes. In MDA-231 and MDA-435 tumors (high metastatic potential), [Lac] was lower compared with MCF and BT474 tumors. [Lac] found to be significantly higher in ER/PR + than ER/PR- and Triple positive than triple negative tumors. We completed the data collection with near completion of lactate quantification with increasing tumor volumes. We have collected the lactate data for MDA-231 and MDA-435 tumors as a function of treatment with targeted drugs and data analysis and correlation with histology is under progress for preparing the manuscripts. We are confident that within the active grant period, all uncompleted lactate data analysis will be completed along with the histology data correlation analysis and manuscripts will be submitted.

References

1. Serganova I, Rizwan A, Ni X, Thakur SB, Vider J, Russell J, Blasberg R, Koutcher JA. Metabolic Imaging: A link between Lactate Dehydrogenase A, Lactate and Tumor Phenotype. *Clin Cancer Res* 17(19):6250-61. Epub 2011 Aug 15.
2. Thakur SB, Yaligar J, Koutcher JA. In vivo lactate signal enhancement using binomial spectral-selective pulses in selective MQ coherence (SS_SelMQC) spectroscopy. Magn Reson Med 2009 Sept; 62(3):591-598.
3. He Q, Shungu DC, van Zijl PC, Bhujwalla ZM, Glickson JD. Single-scan in vivo lactate editing with complete lipid and water suppression by selective multiple-quantum-coherence transfer (Sel-MQC) with application to tumors. J Magn Reson B 1995 Mar; 1 06(3):203-11.
4. Annarao, K. Thomas, N. Pillarsetty, J. Koutcher and S. Thakur. In vivo lactate T1 and T2 relaxation measurements in ER-positive breast tumors using SS1-SelMQC editing sequence, International Society of Magnetic Resonance Medicine, 5-11 May 2012, Melbourne, Australia.
5. Annarao S, and Thakur SB, ‘Quantification of lactate concentrations in orthotopic breast tumors with different growth rates ‘,International Society of Magnetic Resonance Medicine, 5-11 May 2012, Melbourne, Australia.

Appendices: None